EVALUATION OF MORPHOLOGICAL PATTERNS OF ANEMIA IN CHILDREN

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CERTIFICATE

This is to certify that the dissertation entitled "**EVALUATION OF MORPHOLOGICAL PATTERNS OF ANEMIA IN CHILDREN"** submitted by **Dr. M.MUTHURAMAN** to the Faculty of Pathology, The Tamilnadu Dr. M.G.R. Medical university, Chennai in partial fulfillment of the requirement for the award of M.D. Degree in Pathology is a bonafide work carried out by him during the period June 2007 – Nov 2009 under my direct supervision and guidance.

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CONTENTS

INTRODUCTION

It is an axiomatic truth that the children of today are citizens of tomorrow and upon them depend the weal and welfare of the community. In a country like India, children fall an easy prey to anemia as majority of them remain ill-fed, ill-clothed and undernourished due to poverty and ignorance. If not detected at the earliest point of time, this disease which is draconian will spread it's tentacles so widely as to impair or endanger the very physical condition of the children.

Anemia is not a disease but only a manifestation of the underlying disease. Root cause of anemia may be a trivial problem like Iron deficiency anemia, and Vit B12 or folic acid deficiency or it may be the first indication of an ominous disorder like leukemia or aplastic anemia.

Management of pediatric patients depends not only on the hematologist but also on the automated cell counters which give a precise measurement of hematological parameters.

In this study I have evaluated the clinical and hematological profile of pediatric patients coming to pediatric dept, Govt Rajaji hospital who are found to be anemic. The morphological findings are correlated with results of the automated cell counters.

Peripheral smear study, automated hemogram and reticulocyte count done in all children. Serum iron studies, Bone marrow examination Direct Coomb's test, Hb electrophoresis done in children with resistant anemia.

AIM OF THE STUDY

- To evaluate the clinical and hematological profile of pediatric patients with anemia treated in the Department of pediatrics, Government Rajaji hospital, Madurai.
- To classify anemias based on morphology.
- To employ automated hemogram for precise morphological subtyping.
- To evaluate the correlation between etiology and results of automated hemogram.
- To employ special investigations such as Iron studies, Bone marrow aspiration, Hemoglobin electrophoresis in selected cases.

REVIEW OF LITERATURE

ERYTHROPOEISIS

The primitive erythropoeisis arises in the blood islands of the extraembryonic yolksac and shifts to the splanchno-pleural/aorta-gonad mesonephros of the developing embryo. Production then shifts to liver and spleen by about the seventh month of gestation. Bone marrow replaces the liver and spleen as the principal site of production after birth.

In new born, hematopoeitic tissue fills all cavities within the bones and with increasing age it becomes localized to cavities of upper shaft of femur, humerus, pelvis, spine, skull and bones of thorax.

DEVOLOPMENT OF BLOOD CELLS

Blood cells develop from a small population of toti-potent hematopoeitic stem cells which give rise to hematopoeitic cell series.

Red cells are produced by proliferation and differentiation of precursors whose dominant representatives in the bone marrow are the erythroblasts.

During the course of differentiation, the size of erythroblasts progressively decreases, and the character of nucleus and cytoplasm changes as the cells proceed toward the point where proliferative capacity is lost and hemoglobin becomes the dominant protein in the cytoplasm.

The PRO-ERTHROBLAST is the least mature of the morphologically identifiable members of the erythroid series. It has a diameter of 14-20 microns and a basically round outline with minor peripheral protuberances. Nucleus is large, round with fine chromatin, several nucleoli and basophilic cytoplasm.

Proerythroblast gives rise to basophilic erythroblast, a round cell with diameter of 12-16 microns and more basophilic cytoplasm than the pro-erythroblast. The nucleus occupies a relatively large proportion of the cell having coarser and more basophilic chromatin strands.

Next stage is represented by the polychromatic erythroblast, a round cell measuring 12-14 microns. The characteristic polychromatic appearance of the cytoplasm is derived from the mixture of the basophilic RNA and acidophilic hemoglobin. Nuclear chromatin is in coarse, deeply basophilic clumps. Proliferative activity ceases at this stage. Hemoglobin which is found in the cytoplasm possibly gains entrance into the nucleus through pores in the nuclear membrane, and after reaching a critical concentration in the nucleus (20gm/dl) reacts with nucleohistones, thereby bringing about chromosomal inactivation and nuclear condensation.[80,66,82]

Orthochromatic erythroblasts are the last cells of nucleated red cell series. They measure 8-12 microns with a small and pyknotic nucleus with a homogenous blue-black appearance. Active hemoglobin synthesis occurs in the cytoplasm, which contains mitochondria and ribosomes.

Nucleus is extruded from the orthochromatic erythroblast and reticulocytes are formed with diffuse basophilic hue of cytoplasm. Reticulocytes have the same biconcave discoid shape as mature red cells with slightly greater volume and diameter of 8.5 microns.

Reticulocytes enter the circulation and loose their mitochondria and ribosomes over a course of few days and evolve into mature RBCs (Red Blood Cells).

CONTROL OF ERYTHROPOEISIS

Production of red cells is a tightly regulated process. Erythropoiesis is controlled by transcription factors and cytokines, principally GATA-1 and erythropoietin, which influence the rate of lineage commitment, proliferation, apoptosis, differentiation, and number of divisions from the earliest progenitor to late erythroblasts. ERYTHROPOEITIN is a hormone secreted by kidney. Erythropoeitin is a glycoprotein which stimulates the

erythroid precursors resulting in increased red cell production. Maturation takes seven days but this time can be shortened substantially under stress.

STRUCTURE AND METABOLISM OF RED CELLS

Erythrocytes are biconcave disc shaped cells with a mean diameter of 7.2-7.9 microns. They normally lack nuclei and cytoplasmic structures such as lysosomes, endoplasmic reticulum and mitochondria. They exist in large blood vessels as biconcave discs, but change their shape to parachute like conformation in small capillaries with diameter less than that of red cell.

The membrane of the RBC is a highly deformable, but non-expansile or contractile structure. It's integrity is firmly maintained by the attachment of it's inner surface to a lattice like structure of specialized cytoskeletal proteins which supports the membrane and dictates the shape of the erythrocyte.

The major protein component of the membrane is band 3 protein which spans the full width of membrane and encases channels which facilitates transport of glucose and anions.

Another important transmembrane protein is glycophorin.

The exterior surface of this molecule is heavily substituted with sugars containing sialic acid which contributes to the negative charge of the outer surface of red cell at physiological pH. Glycolipids in the outer leaflet of membrane contain specific oligosaccharide sequences which constitute the ABO blood group substances. Phospholipid and cholesterol are the dominant lipid components of the matrix of the membrane.

The most important constituent of the cytoskeleton is the protein SPECTRIN. Inter twined spectrin molecules are linked together by a specific protein and actin to form a lattice like network which is attached to the inner surface of the membrane. This network is a resilient structure which normally causes red cells to resume the biconcave disc form after forces causing distortion are removed.

Specific sites on spectrin serves as points of attachment to molecules that protrude from the membrane (band 4.1 protein) and to the protein ankyrin which links the spectrin to the internal pole of the band 3 protein.

Hemoglobin

Hemoglobin molecules consist of two pairs of polypeptide globin chains. Each globin chain bears a haem group whose central iron atom is the site at which oxygen attaches to hemoglobin.

The type of globin chain synthesized by erythroid precursors undergoes progressive change with time after conception .The embryonic hemoglobins Hb Gower 1 & 2 and Hb Portland predominate until third month of gestation after which fetal Hb becomes the major form.

HbF is the primary hemoglobin found in the fetus. Fetal hemoglobin consists of two ά and γ globin chains. It has a higher affinity for oxygen than adult hemoglobin, thus increasing the efficiency of oxygen transfer to the fetus. The relative quantities of HbF rapidly decrease to trace levels by the age of 6 to 12 months and are ultimately replaced by the adult forms, HbA and HbA2.

Adult Hemoglobin consists of two alpha (ά) and two beta (β) globin chains. Significant amount of adult hemoglobin is synthesized during third trimester of gestation. The proportion of adult Hb progressively increases to 25% of total hemoglobin at birth, and about 97% 12 months later. Another minor form of adult hemoglobin is HbA_2 which is

made up of two ά and two δ globin chains. It is present only in trace amounts in the fetus and does not exceed 2% of total hemoglobin in normal adults.

ERYTHROCYTIC INDICES

MCV(Mean corpuscular volume)

Mean corpuscular volume is the average volume of a red blood cell.

MCV is a useful red cell index in classification of the anemias.

MCV=Hct (L/L) X 1000/red cell count (10^{12}/L).

The MCV is measured in femtolitres (fl, 10^{-15}L).

MCH (Mean corpuscular hemoglobin)

MCH is a measure of the average hemoglobin content per red cell.

MCH=hemoglobin (g/L)/red cell count (10^{12}/L).

MCH is measured in pg.(10^{-12}gm).

MCHC (Mean corpuscular hemoglobin concentration)

MCHC is the average concentration of hemoglobin in a given red cell volume. MCHC may be calculated by the following formula

MCHC-Hb (gm/dl) /Hct (L/L).

Expressed in gms/dl.

ANEMIA

Anemia was defined as a hemoglobin concentration of at least 2 standard deviations lower than age- and sex-specific average. In routine practice anemia is defined as the reduction below normal in the volume of packed red cells or a reduction in Hb concentration of blood.

WHO expert group[90] proposed that anemia should be considered to exist when Hb is below 11 grams% in children aged 6 months to 6 years and 12 gms % in children aged 6-12 years.

At all ages normal MCHC is 34.

Severity of the anemia can be graded as

Mild anemia – 10-11gms[77,]

Moderate anemia – 7-10gms%,

Severe anemia < 7gms %[71]

CLASSIFICATION OF ANEMIA [92]

Anemias are classified on the basis of red cell morphology as microcytic hypochromic, normocytic normochromic and macrocytic anemias. The main advantage of classification is it is simple, based on readily available red cell indices and forces the physicians to consider the

most important types of curable anemia such as iron deficiency anemia, and anemia due to vitamin B12 and folic acid deficiency.

Such practical considerations has led to the wide acceptance of this classification.

Microcytic hypo chromic anemia

RBC are smaller in size with exaggerated central pallor. The MCV is less than 75 fl and MCH less than 24 pg respectively. Common causes are iron deficiency anemia (IDA), thalassemias anemia of chronic disease and sidero blastic anemias.

Normo cytic normo chromic anemias

The RBC have normal size and hemoglobinization in the blood films. MCV, MCH are within normal limits. Causes are red cell membrane defect, enzyme defects, acquired hemolytic anemias due to anti bodies, microangiopathic hemolytic anemias, acute red cell loss, hyper splenism and chronic kidney disease.

Macro cytic anemia

The RBC are larger in size and lack central pallor.

MCV, MCH increased and MCHC is with in normal limits.

Common causes are Vitamin B12 and Folic acid deficiency. Aplastic anemias, Hypothyroidism, Liver disease and Bone marrow infiltration can also present as macrocytic anemia.

EPIDEMIOLOGY

Anemia is a global health problem and hypochromic microcytic anemias due to iron deficiency is the most common nutritional disease prevalent in developing countries

80 % of children in developing countries and 20% of children in developed countries are anemic according to MARTIN PL et al[54] .Prevalence of anemia in children is 51% and girls had a higher prevalence of anemias in another study[86]. Nearly the half of well nourished children are anemic (47.6%[86]). The NFHS survey (National Family Health Survey) II (1998-99) estimated the prevalence of anemia as 74% among children aged 6 months-3 years and severe anemia as 1.3%. (Hb<7 gm/dl).[39] Kapoor D et al and padmanaban A et al estimated the incidence of anemia in children at 64% and 37.9-45.1% respectively[46,67.]

In infants and children the most common cause of anemia is micro cytic hypo chromic anemia, accounting for 55.4 – 64% of total anemias[86,64,7.]. The prevalence of iron deficiency anemia in the United

States ranges from 3 to 10 percent and may be as high as 30 percent in low-income populations. Azmath manzoor et al[7] put the prevalence of iron deficiency anemia among total anemias at 62.9 %.

D Viswanath et al and Alukh et al revealed that 89% and 64% of children with Microcytic hypochromic anemia had Iron Deficiency anemia.[87,5].

Prevalence of thalassemias among communities of Punjabis, sindhis, gujaratis and parsis. India has an estimated four crore thalassemia carriers and 10,000 thalassemia major children are born in India every year. Prevalence of thalassemia gene in general population varies from 2.7% in Mumbai, 5.5% in Delhi, and 10.4% in kolkata.[6.] Incidence of Thalassemia major and Thalassemia minor among anemic children varies from 4.7% to 14.34%. Incidence of G-6PD (Glucose 6 Phosphate dehydrogenase) deficiency is estimated to be 2.6% in India[68].

The prevalence of VitB12 deficiency and combined Vit B12 and folate deficiency is estimated as 36.6% and 2.2% respectively. Prevalence of macrocytic anemias due to VitB12 and folic acid deficiency is 2.2%[29].

PATHOPHYSIOLOGY OF ANEMIAS

The underlying causes for anemias vary from nutritional deficiency to inherited enzyme defects. The most common cause of Microcytic hypochromic anemia is Iron Deficiency anemia. For normal iron balance 1mg of iron must be absorbed from diet everyday. Iron deficiency anemias can result from dietary lack of iron, impaired absorption, increased requirement and chronic blood loss.

In developing countries helminthic infestations, malaria and malnutrition are also common causal factors.[22]In developed countries introduction of unmodified cow's milk and defective absorption are also implicated. [85,31,74.]

Three pathogenic factors are implicated in the anemia of iron deficiency. First, hemoglobin synthesis is impaired as a consequence of reduced iron supply. Second, there is a generalized defect in cellular proliferation. Third, survival of erythroid precursors and erythrocytes are reduced .Iron deficiency leads to decreased transferrin saturation which in turn results in reduced iron supply to marrow erythroid precursors. As a result, the amount of free erythrocyte protoporphyrin increases and each

cell produced contains less hemoglobin resulting in microcytosis and hypochromia.

As the total body iron levels begins to fall, a characteristic sequence of events ensues.

The first stage also known as prelatent stage represents a reduction in iron stores without reduced serum iron levels.[12,36]. This stage is usually detected by low serum ferritin measurement. Next stage is latent stage in which iron stores are exhausted but blood hemoglobin levels remain normal.[36,18,26,47,84] Reduced transferrin saturation, increased TIBC (Total iron binding capacity), increased free erythrocyte protoporphyrin seen and MCV is within normal limits. In the third stage, blood hemoglobin levels fall below normal and iron deficiency anemia is apparent.

As Iron deficiency progresses morphological changes in RBCs follow development of anemia. The production of hypochromic microcytic RBCs is primarily attributed to delay in the synthesis of Hemoglobin. During erythropoeisis Hb enters the nucleus and it reaches a critical concentration in the nucleus and reacts with nucleohistones causing nuclear condensation and chromosomal inactivation. Microcytic cells are produced in iron deficiency because it takes longer to reach the critical

hemoglobin concentration and the generation time is unaffected. Hence more cell divisions occur before nuclear inactivation and the resulting cell is small[80,66,82].

Impaired red cell production occurs in association with chronic diseases like osteomyelitis, lung abscess, rheumatoid arthritis and bacterial endocarditis which closely resembles Iron deficiency anemia. In these conditions there is some impediment in the transfer of iron from the storage pool to the erythroid precursors. In addition, marrow erythroid progenitors do not proliferate adequately because erythropoeitin levels are inappropriately low for the degree of anemia.

The reduction in renal erthropoeitin generation is attributed to production of IL-1 (Interleukin-1), tumor necrosis factor (TNF) and interferon-γ. These cytokines also inhibit the release of iron from the storage pool. The anemia is usually mild and the dominant symptoms are those of the underlying disease. The red blood cells can be normocytic and normochromic or hypochromic and microcytic as in anemia of iron deficiency. Presence of low serum iron and reduced total iron-binding capacity in association with abundant stored iron in the mononuclear phagocytic cells are the common features.

The thalassemia syndromes are a heterogenous group of inherited disorders caused by genetic lesions leading to decreased synthesis of either the α or β globin chain of HbA. The hematologic consequences of diminished synthesis of one globin chain stem not only from low intracellular hemoglobin, but also from relative excess of the unimpaired chain.Beta-thalassemia is caused by deficient synthesis of betachain due to defect in single beta-globin gene on chromosome 11.

Based on severity there are 3 types;

Thalassemia major-total absence of beta-globin chains in homozygous state, caused by mostly point mutations of beta-globin gene.

It results in severe anemia.

Thalassemia minor-Heterozygotes with one beta-thalassemia gene and one normal gene. Usually aymptomatic, mild microcytic anemia will occur**.**

Thalassemia intermedia-which is genetically heterogenous with moderate to severe anemia.

Two mechanisms are proposed in pathogenesis of anemia in thalassemias. The deficit in HbA synthesis produces under-hemoglobinised hypochromic microcytic red cells. Another important factor is diminished

red cell survival because of cell membrane damage due to insoluble inclusions of free alpha chains. The inclusion bearing erythroid precursors are prone to intramedullary death and escaping red cells are killed by splenic sequestration and destruction.

Alpha-thalassemia is caused by deficient synthesis of alpha-chain which is encoded by pair of genes on chromosome[16]. Reduced synthesis of alpha chain is due to deletions of alpha-globin genes.

In newborn with αthalassemia, excess unimpaired γ globin chains forms γ_4 known as Hb-Barts. In adults, excess β-globin chains form HbH,which precipitates in oxidized form in older red cells which are then removed by splenic macrophages.

In sideroblastic anemias,there is decreased production of protoporphyrin or impaired incorporation of iron into protoporphyrin in the erythroid cells. This results in insufficient heme generation and excess iron accumulation in the marrow. Patients have excess body iron and serum ferritin and hypochromic microcytes in the peripheral smear. For confirmation bone marrow examination is needed which will show ringed sideroblasts.

Pathogenesis of hemolytic anemias may be inherited or acquired.

Hereditary spherocytosis is the most common hereditary hemolytic anemia. The pathophysiology behind hereditary spherocytosis is mutations that affect the cytoskeletal proteins such as spectrin, band3-protein, ankyrin which leads to reduced membrane stability. These red cells when exposed to shear stresses in circulation loose membrane fragments which forces the cell to assume the spheroid shape. These spherocytes are trapped and destroyed in spleen resulting in anemia.

Alloimmune hemolytic disease of newborn is due to destruction of baby's red cells by maternal IgGAbs. Antibodies are directed against the Rhesus or ABO blood group antigens in baby's RBCs. Basic mechanism of Autoimmune hemolytic anemias is derangement of mechanisms of immunological tolerance. Antibodies are produced that are directed against our own red cells which produces destruction of red cells with or without fixing complement. The antibodies are warm antibody or cold agglutinin or cold hemolysin.

Megaloblastic anemias occur due to VitB12 and folic acid deficiency predominantly.VitB12 deficiency occurs due to inadequate diet or due to pernicious anemia and gastrectomy, whereas folic acid deficiency

occurs due to tropical or celiac sprue and Ileal resection. Deficiency or impaired metabolism of VitB12 or folic acid which are coenzymes required for synthesis of thymidine leads to defective or deranged DNA synthesis, with an attendant delay or block in cell division. Nuclear-cytoplasmic asynchrony occurs as cytoplasmic maturation proceeds in advance of nuclear maturation.

MANAGEMENT OF ANEMIA IN CHILDREN

Anemia is an important cause of morbidity in children which may be a clue for an underlying life threatening illness.

Proper history from parents, detailed physical examination and lab investigations are helpful in identifying the cause of anemia.

Iron deficiency anemia (IDA) is rare in infants less than 6 months of age particularly in breast fed children as mother's milk contains more iron than cow's milk. Peak prevalence of iron deficiency anemia occurs during late infancy and early childhood when there is rapid growth and low levels of dietary iron. Mucosal pallor is a common feature of severe anemia.

PICA (eating non-edible substances) points towards iron deficiency anemia. Koilonychia (spooning of nails) was a common sign in the past but is now rarely encountered. Children suffering from Thalassemia Major have enlarged skull bones and frontal bossing with depressed nose.(Fig 21&22) Jaundice indicates a hemolytic process. A family history of anemia, splenomegaly, jaundice, gall stones point towards congenital hemolytic anemia. Splenomegaly is also seen in malignancy or hypersplenism. Petechiae, purpura can be seen in acute leukemias or immune thrombocytopenic purpura.

Careful evaluation of a well-prepared blood smear (Fig 1&2) is an important part of evaluation of anemia[93].Though data obtained from automated hematology analysers permit a specific diagnosis in some cases abnormal cellular morphology can be appreciated only in peripheral smear examination. If anemia is associated with thrombocytopenia or leucocytosis or the presence of blasts in peripheral smear it denotes the possibility of bone marrow failure due to leukemia or aplastic anemia or hypersplenism.

Next step is to assess the size, shape, color of RBCs. Cells with small size and exaggerated central pallor (microcytic hypochromic) seen in Iron deficiency Anemia (Fig 4), thalassemias. Polychromatic large RBCs (Fig 11) are indicative of reticulocytosis. Spherocytes which are spherical, small cells with absent central pallor (Fig 10) are seen in hereditary spherocytosis and immunehemolytic anemias.

In sickle cell anemia, sickle cells which are bipolar, spiculated forms pointed at both ends are seen. Distorted, fragmented red cells (schistocyte) in peripheral smear is an evidence of microangiopathic hemolytic anemias and it is also formed in prosthetic valves and severe burns.

Acanthocytes (spur cells) with varying cytoplasmic projections and dense centre are seen in abetalipoproteinemia, parenchymal liver disease and postsplenectomy children. Red cells with short evenly spaced spicules known as burr cells are encountered in children with uremia.

Reticulocyte count

Reticulocyte count provides an initial assessment of the cause of anemia.Reticulocyte count is decreased in impaired RBC production and elevated in anemias due to loss in peripheral circulation. Reticulocyte count normally is 0.5%-1.5% of total RBCs.

Absolute reticulocyte count-25-75X10^3/cumm.[94]

Anemia with low reticulocyte count may be due to reduction in red cell precursors (hypogenerative) or it may be due to ineffective erythropoeisis with erythroid hyperplasia in bone marrow.

MCV

MCV tends to be the single most useful measurement for analysis of causes of anemia with low reticulocyte count.[10,27,52.]

MCV and MCH correlate closely. [25,34.]

MCV less than 75fl denotes microcytic anemia. [,62]

MCH less than 24pg is indicative of hypochromia.

MCHC is a measure of cellular hydration status. A high value (>37gm/dl) is seen in hereditary spherocytosis, HbC and homozygous sickle cell anemia children.

Low MCHC levels are seen in Iron deficiency anemia[59].

APPROACH TO MICROCYTIC ANEMIA

In children common causes of microcytic hypochromic anemia are Iron deficiency anemia, Thalassemia and rarely anemia of chronic disease. Lead poisoning, sideroblastic anemias cause microcytosis but are rare in children. Iron deficiency anemia is the most common cause of Microcytic hypochromic anemia.[65,30].

Reticulocyte count is decreased in Iron deficiency anemia and Anemia of chronic disease and it is increased in α or β thalassemia or HbE disease.

RBC count is elevated in (>5 million/cumm) in children with Thalassemia trait and depressed in children with Iron deficiency anemia..In Thalassemia minor children microcytosis is severe than might be expected for the degree of anemia. Target cells (Fig 9) and basophilic stippling tend to be more prominent in thalassemia than Iron deficiency anemia.

Modification of mentzer index can be used to differentiate Iron deficiency anemia and Thalassemia Trait[58] which is based on MCV and RBC count.

MCV/RBC count >14 –suggestive of Iron Deficiency anemia

MCV/RBC count<12- suggestive of Thalassemia trait disorders.

Red cell distribution width (RDW) is a useful parameter to differentiate Iron deficiency anemia from other causes.RDW measures the anisocytosis derived from erythrocyte volume distribution.[23,9,44]

Anisocytosis is an early and prominent finding in Iron deficiency anemia and is the first parameter to rise even before appearance of anemia. An increased RDW appears to be 90-100% sensitive for Iron deficiency and 50-70% specific.[57]Increase in red cell distribution width correlates with severity of Iron Deficiency anemia.

Homozygous hemoglobinopathies HbE and HbC tend to be microcytic and normochromic and many target cells are apparent in blood smear.[17,24]

Homozygous β thalassemia cases show extreme anisopoikilocytosis and nucleated red cell precursors (Fig.12) and target cells in peripheral smear apart from signs of hemolysis and ineffective

erythrpoeisis with consequent erythroid hyperplasia in bone marrow (Fig.16).

A therapeutic trial of oral iron is an appropriate initial diagnostic test for microcytic anemias with features suggestive of Iron Deficiency anemia in Peripheral smear and automated hemogram.[62]

Further investigation is necessary only if there is no response to treatment. A dose of 6mg/kg/day of elemental iron given in the form of oral ferrous sulfate is the preferred method.[62,] Reticulocyte count should increase in 5-10 days and the serum Hb should increase by 1gm/dl/week thereafter. Poor response is due to factors like poor compliance, poor absorption, incorrect diagnosis and ongoing blood loss. Further lab analysis is needed in children who are not responding to rule out other causes.

Tests for screening Iron deficiency Anemia are Serum iron, TIBC (Total Iron Binding capacity), serum ferritin and free erythrocyte protoporphyrin. Serum Iron levels are measurement of Iron bound to transferrin. TIBC is an indirect measurement of transferrin in terms of amount of iron it will bind. Serum Iron has considerable fluctuation in values [52,19,79,40] of individual upto 10-40% within a single day or from day

to day.[78,89] TIBC values show only slight variation. Normal serum iron varies from 70-200 µgm/dl TIBC value varies from 250-435µg/dl. Transferrin saturation is 20-45% normally. Values below 16% are noted in Iron Deficiency and Anemia of chronic disease. TIBC is often increased in Iron Deficiency and decreased in Anemia of chronic disease.[91]

Determination of serum ferritin is often the only test needed to diagnose Iron Deficiency anemia. Serum ferritin concentrations is proportional to total body iron stores and are not influenced by recent iron therapy.[96] Ferritin values varies from 10-500 µg/l Sr ferritin is low in Iron Deficiency anemia,(<10µgm/L) ,normal in thalassemia and high in Anemia of chronic disease.[51]Sr ferritin can be elevated in infection,inflammation or malignancy as it is an acute phase reactant.[38]

Combined use of soluble transferrin receptor level and (sTfR) and the sTfR/log ferritin index is useful in these situations.[15]The sTfR is increased in erythroid hyperplasia of marrow such as IDA, thalassemia and low or normal in inflammation and malignancy.[3,20,70.]The sTfR/log ferritin index is elevated in Iron deficiency but not thalassemia.[20,83]The reticulocyte Hb content (CHr) is an important test in identifying iron deficiency at the earliest in children particularly infants. CHr can detect 83% of IDA

whereas transferring saturation can detect only 26% of early cases, CHr levels <26 pg denotes anemia.[14,1]

Soluble transferring receptor –ferritin ratio is an indicator of iron status.

Free Erthrocyte protoporphyrin (FEP) is accumulated in RBCs when iron is unavailable or unable to combine with protoporphyrin to form heme. It is useful in differentiating Iron deficiency anemia (Elevated FEP) from thalassemia minor(normal FEP)[75]

Evaluation of bonemarrow iron stores is used much less nowadays. In Bone marrow aspirates, hemosiderin appears as blue granules in Prussian blue staining.[69.]

Normal marrow iron is graded 1+ to 3+.In Iron deficiency anemia, marrow hemosiderin is absent .In Anemia of chronic disease, iron is present with grade2-3.Iron stores are greatly increased in thalassemia major(5+--6+). Hb electrophoresis should be obtained in patients with microcytosis and target cells with normal serum ferritin levels.

Beta-thalassemia major children have elevated HbF levels ranging from10-90% and HbA2 levels may be normal or elevated,(5-7%) Hb A2 is elevated in heterozygous carriers of β Thalassemia varying from

3.5-7%.[88.]Thalassemia trait causes microcytosis with mild anemia and Hb electrophoresis reveal HbBarts (γ4 tetramers) in new born period.

In HbH disease electrophoresis shows HbH-25%, Hb Barts and low normal levels of HbA2.

APPROACH TO NORMOCYTIC ANEMIA

Normocytic anemias are those with MCV within normal limits (75-96fl) First step in evaluating normocytic anemia is to look for pancytopenia, which suggests ineffective hematopoeisis affecting all lineages. Peripheral smear will show anisopoikilocytosis, nucleated red blood precursors, blasts and decreased platelets.

A bone marrow biopsy or aspirate is indicated to rule out severe aplastic anemia, leukemia or infiltration by metastasis or storage disease (Gaucher's disease). Hypercellular marrow in the presence of pancytopenia indicates hypersplenism. If pancytopenia is absent, the reticulocyte response should be assessed. Low reticulocyte count with normal red cell morphology, Hb>8 gm/dl, elevated serum ferritin and ESR are evidence of anemia of chronic disease. Liver diseases, ndocrinopathies, renal insufficiency can also present with same picture.

Normocytic anemias with elevated reticulocyte count suggests premature destruction of RBCs due to hemolysis or blood loss.[56,28] The source of blood loss may be occult or overt. It may be acute or chronic, congenital or acquired, intrinsic or extrinsic to RBC, intravascular or extravascular.

Intravascular hemolysis can manifest as increase in plasma Hb, decreased serum haptoglobin and the presence of hemoglobinuria[33].Most hemolytic diseases are associated with extravascular hemolysis. They are caused by intrinsic defect of red cell itself or caused by extrinsic agents acting on normal RBCs. Most intrinsic ones are inherited whereas extrinsic ones are acquired.

An elevated MCHC (>37gm/dl) and increased osmotic fragility test coupled with an RDW>14 is diagnostic of hereditary spherocytosis.[60] G-6PD deficiency patients have increased serum bilirubin, hemoglobinuria with schistocytes or spherocytes in peripheral smear. With supravital stains such as brilliant cresyl blue we can demonstrate Heinz bodies.

Immune mediated destruction to RBCs is confirmed by a positive direct or indirect coomb's test. Spherocytes are seen in blood smear. In neonates, immune mediated hemolysis is due to placentally transferred

maternal antibodies due to sensitization to Rh antigen or a response to other blood group antigens.

Mechanical damage to the red blood cells occur in cardiac hemolytic anemias and microangiopathic hemolytic anemias. In the peripheral smear we can see fragmented, contracted, triangular and helmet shaped forms or microspherocytes In hemolytic uremic syndrome, severe anemia is accompanied by neutropenia and thrombocytopenia. Reticulocytosis, fragmented red cells, microspherocytes can be seen in peripheral smear. Hemoglobinemia, hemoglobinuria and reduced haptoglobin and increased lactate dehydrogenase levels are seen. Direct antiglobulin test is negative.

APPROACH TO MACROCYTIC ANEMIAS

Morphologically and biochemically macrocytic anemias are divided into megaloblastic macrocytic and non-megaloblastic macrocytic anemias. Most commonly VitB12 or folate deficiency and less commonly inherited or drug induced disorders of DNA synthesis can cause megaloblastic anemias. Aplastic anemias, congenital or acquired, hypothyroidism, liver disease are causes of non-megaloblastic macrocytic anemias.

Peripheral smear examination is most helpful in these classification of macrocytic anemias. In megaloblastic anemias, macro-ovalocytes and hypersegmented neutrophils and giant platelets may be seen (Fig 5). In non-megaloblastic anemias macrocytes are seen with no detectable abnormality in white blood cells (WBC) or platelets.

Neutrophil hypersegmentation is the most sensitive sign of megaloblastic anemia.98% of megaloblastic anemia showed atleast one six lobed neutrophil per 100 WBCs.[74]Macroovalocytes are well filled with Hb with reduced or absent central pallor.

In megaloblastic macrocytic anemias, RDW is substantially increased which occurs much earlier than appearance of anemia[8].Bone marrow examination is essential for diagnosis of megaloblastic anemias. Marrow is hypercellular with characteristic large size megaloblasts. These cells show a large nucleus with particulate or sieve like chromatin (Fig 17). Orthochromatic normoblasts can show megaloblastic changes with giant metamyelocytes in leucopoiesis can be seen. Sr VitB12 levels and folate levels are measured to distinguish megaloblastic anemias due to VitB12 or folate deficiency. Vit B12 levels can be determined by microbiological assay or by competitive isotope binding techniques.[2,55,49] Serum and

erythrocyte folate levels may be determined microbiologically or by Isotope dilution[35,37,45,81.] Erythrocyte folate levels are a better index for tissue folate stores.

Measurement of methylmalonic acid and homocysteine may serve as sensitive indicators of vitB12 or folate deficiency.In aplastic anemias peripheral smear shows thrombocytopenia and leucopenia along with macrocytes and reticulocytopenia.

Bone marrow aspirate shows aplastic marrow with dysplastic features. Diagnosis of fanconi's anemia is based on demonstration of increased chromosomal breakage in the presence of DNA cross linking agents such as Mitomycin-C (MMC) or Diepoxy butane.[73,4]

MATERIALS AND METHODS

The present study was carried out in the department of pathology, Madurai medical college, Madurai between August 2007-July2009.All children less than 12 years of age attending pediatric OP clinic and found to be anemic were enrolled in this study.

Study design-Prospective study

Collaborating dept-Pediatrics Department, GRH Madurai.

Children on Iron medications, who were given blood transfusions within three months were excluded from this study. Informed consent obtained from parents of children.

A thorough history, complete physical examination was done followed by evaluation of hematological parameters using automated hemogram.

The results of 248 children were shown in this study.

Peripheral smear study, reticulocyte count were done in all cases.

Bone marrow aspiration, Direct Coomb's test, osmotic fragility test, Serum Iron, ferritin studies, Hb electrophoresis were done in selected cases.

SAMPLE COLLECTION AND PROCESSING

Smears are dried and stained by leishman's stain containing eosin as the acidic stain and the methylene blue as the basic stain.

Leishman's stain prepared by dissolving 0.15gms of powdered stain in 100% pure (acetone-free) methyl alcohol.

Pour 8-12 drops of leishman's stain over the slide and wait for two minutes. Then add double the volume of buffer water (16-24 drops) and wait for 8-10 minutes. Then wash the slide in running water for 2-3 seconds. Wipe off the excess stain from undersurface of the slide and air dry smear. Then the smear is examined under the microscope.

Reticulocyte count

Procedure-Take 2-3 drops of blood anticoagulated with either EDTA or balanced oxalate in a small test tube. Then add 2-3 drops of supravital stain (brilliant cresyl blue or new methylene blue). Mix well and then incubate the mixture at 37'C for 15-20 minutes. Mix again and then prepare a smear for examination.

Reticulocytes are identified by dark blue granules in cytoplasm. One thousand RBCs are counted and the number of reticulocytes seen during that count are reported as a percentage of total number of RBCs.

AUTOMATED HEMOGRAM

Automated hemogram in our clinical pathology lab is the SYSMEX KX-21 cell counter(Fig 20).Cell counters enumerates cells in a small

aperture by measuring changes in the electrical resistance as the cell passes through the aperture.

This principle is called APERTURE-IMPEDANCE METHOD.

Hemograms print data in numerical forms as well as give histograms of blood cell size. Data generated include a three part white cell differential (absolute count, percentage) in addition to red cell counts, WBC count, platelet count, Hb, hematocrit, MCV, MCH, MCHC, RDW, MPV (Mean platelet volume) and flags abnormal cell populations including blasts and atypical cells.

BONE MARROW EXAMINATION

Marrow aspiration is done by aspirating marrow from the iliac crest or upper end of tibia. Smears are stained with leishman's stain and assessed for cellularity, maturation pattern of erythroid and myeloid series and the presence of megakaryocytes (Fig 13,14,15)

The pattern of erythroid maturation is classified as micronormoblastic, normoblastic or megaloblastic.

SPECIAL STAINS

Staining for hemosiderin in bonemarrow can be done using Perl's stain.

Principle – Ionic Iron reacts with acid ferrocyanide to give a blue colour.

Reagents- 4% Hcl, 4% potassium ferrocyanide,basic fuchsin.

Equal volume of 4% Hcl and 4% potassium ferrocyanide are taken in a jar heated to 56'C and slides are kept in the solution for 30 minutes. Slides are then washed in tap water. Counterstain with basic fuchsin stain for 5 minutes. Slides are then immersed in absolute ethanol and tap water. Hemosiderin in macrophages can be seen as blue staining material within bone marrow fragments. Erythroid precursors also show hemosiderin in the form of small blue granules in cytoplasm.(Fig18)In Iron deficiency anemia, there will be no stainable iron in marrow.

OSMOTIC FRAGILITY TEST

Patient's red cells are placed in series of increasingly hypotonic saline solutions to measure the osmotic fragility of RBC.

Stock solution of buffered sodium chloride osmotically equivalent to 10% Nacl is prepared and working solutions are prepared from the stock solution. 0.85.0.75, 0.65, 0.60, 0.55, 0.50, 0.45, 0.40, 0.35, 0.30, 0.20, 0.10 percent NaCl solutions are prepared. Add 0.05 ml of blood to 5 ml of series of hypotonic solutions and mix well and allow the tubes to stand at room temperature for 30 minutes. Remix, and then centrifuge for 5 minutes at 2000 rpm. The amount of hemolysis in each tube is measured by reading

the absorbance of supernatant solution in photoelectric colorimeter or a spectrophotometer.

Normal RBCs start to hemolyse at 0.45% and complete hemolysis occur at 0.3%.In spherocytic anemia, RBCs show increased osmotic fragility and they start to hemolyse at 0.6% and end at 0.45%.

COOMB'S TEST

Coomb's serum prepared by injecting human serum into rabbits and collecting the anti-human globulin antibodies produced by rabbits. Indirect Coomb's test done by mixing two drops of test serum with 5% saline suspension of red cells and incubating for one hour. Then check for agglutination. Direct Coomb's test performed by adding one drop of Coomb's serum to 2-3 drops of red cells and incubating for 5 minutes. Then centrifugation done and we have to look for agglutination.

Hb Electrophoresis

Most useful method for detection of abnormal Hemoglobins such as Hemoglobin C, D, E and Hemoglobin S. The principle is Hemoglobins are separated on a variety of supporting media on the basis of electrical charge differences.

Cellulose acetate electrophoresis at pH 8.6 is the method of choice.

Agar gel electrophoresis using a citrate buffer at pH 6.0 (Fig.19) is useful in supplementing the information gained from other methods, as the mobility of some abnormal hemoglobins on agar gel differs from that on other supporting media.

DATA ANALYSIS;

The information collected regarding all the selected cases were recorded in a Master Chart. Data analysis was done and percentage, mean, standard deviation, x^2 and 'p' values were calculated. A 'p' value less than 0.05 is taken to denote significant relationship.

Sensitivity and specificity were calculated using the following formulae.

$$\text{Sensitivity} = \frac{\text{True positive}}{\text{True positive + False negative}} \times 100$$

$$\text{Specificity} = \frac{\text{True negative}}{\text{False positive + True negative}} \times 100$$

$$\text{Accuracy} = \frac{\text{True positive + True Negative}}{\text{No. of cases}} \times 100$$

OBSERVATION, ANALYSIS AND RESULTS

In the study period from January 2008 to June 2009, 3381 children with anemia were referred to clinical pathology laboratory for investigations. Among them 1914 children were boys and 1467 were girls.

Anemia was found in 614 children .Of these children, 248children were enrolled in this study after carefully examining the clinical history and excluding the children who were undergoing treatment.

Out of these 248 children, 152 were boys and 96 were girls. (CHART 1) Morphologically 147 children had microcytic hypochromic anemia, 24 children had dimorphic anemia and 70 children had normochromic normocytic anemia and 7 children had macrocytic anemia. (CHART 2)

The overall incidence of microcytic hypochromic anemia was 59.25%, dimorphic anemia was 9.6%, normocytic anemia was 28.25% and macrocytic anemia was 2.8%.

CHART 1

SEX DISTRIBUTION

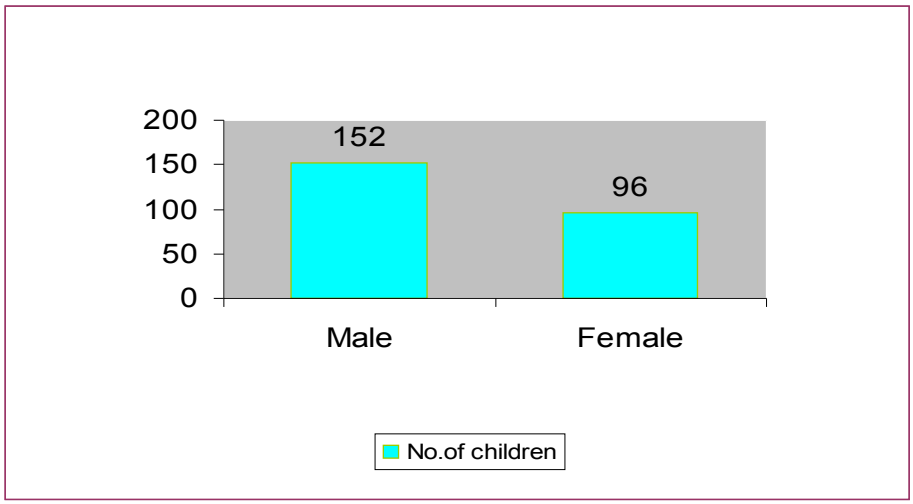
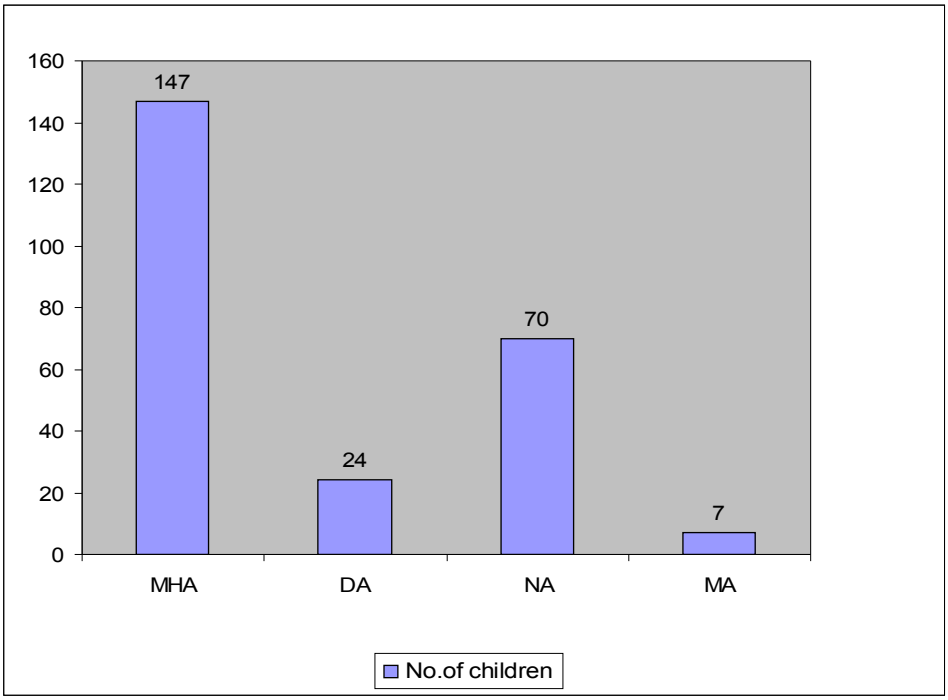


CHART 2

MORPHOLOGICAL PATTERNS OF ANEMIA



Among microcytic hypochromic anemia, Iron deficiency anemia was seen in 129 children (87.7%), Thalassemia major -12 children (8.1%) and thalassemia trait in 4 children (2.7%) and HbE and Anemia of chronic disorder in one child each.(1.5%) In dimorphic anemia, ,Iron deficiency anemia was present in 19 children (79.1%) and Anemia of chronic disorder in 5 children.(20.9%).In normocytic anemias ,Iron deficiency anemia was seen in 29 children(41.4%) , Acute leukemia in 19 children (27.1%) ,Immune thrombocytopenic purpura-4 children(5.7%),Anemia of chronic disorder in 3 children (4.2%), Immune hemolytic anemias in 10 children (14.8%), storage disorders in 2 children. In macrocytic anemia, aplastic anemia seen in 5 children (71.4%) and megaloblastic anemia in 2 children (28.6%) (Chart 3).

177 children were affected with Iron deficiency anemia (71.37%). Acute leukemia was the underlying cause of anemia in 19 children (7.66%).

Thalassemia major diagnosed in 12 children (4.83%) and four children had (1.62%) thalasemia trait. Anemia of chronic disorder was the cause in 9 children (3.62%) and Immune hemolytic anemia was seen in 10 children (4.03%).

Hereditary spherocytosis and storage disorders were seen in 3 and 2 children respectively. HbE was present in one child. Immune thrombocytopenia was the underlying cause in 4 children.

A plastic anemia and megaloblastic anemia contributed to 5 and 2 children respectively. (CHART 4).

CHART 3

RELATIONSHIP BETWEEN MORPHOLOGY AND ETIOLOGY

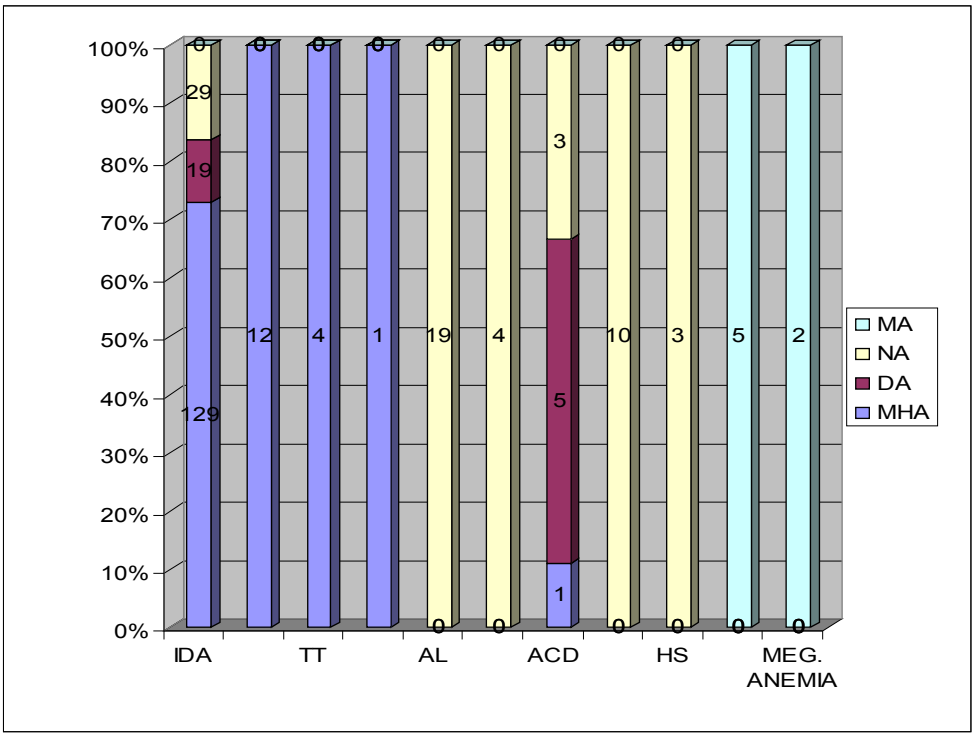
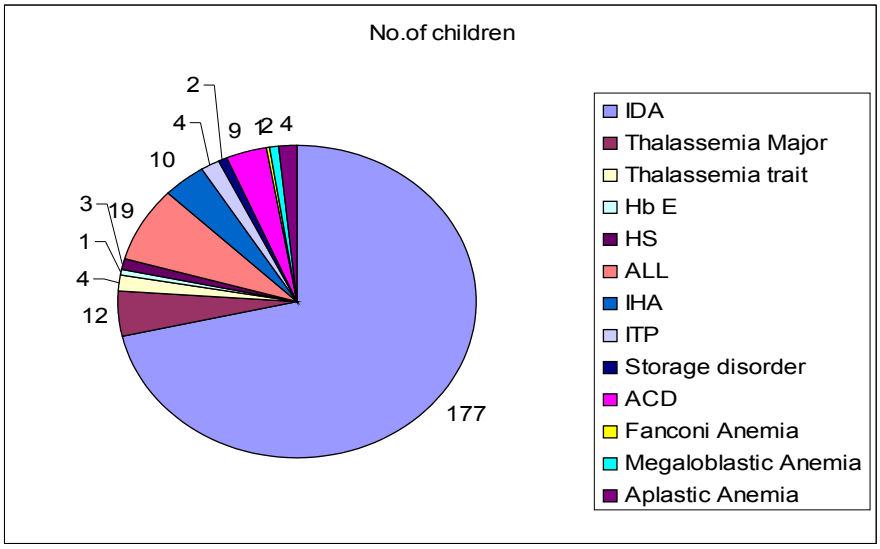


CHART 4

ETIOLOGY OF ANEMIA



AGE

In our study, mean age was 4.4 years. 52 children were infants (20.9%). 78 children were aged between 1-3 years (31.4%). 52 children were aged between 3-6 years (20.9%) and 66 children had ages varying between 6-12 years (26.8%) (CHART 5). In Infants, 35 had Iron deficiency Anemia, 6 infants had Thalassemias, one had acute leukemia and 7 infants had immune hemolytic anemia. Aplastic anemia and hereditary spherocytosis were present in two and one children respectively. Among children aged between 1-3 years, Iron Deficiency anemia found in 63 children.

Thalassemias presented in 4 children, 5 children had acute leukemia. One child of Immune hemolytic anemia, 3 children with Anemia of

chronic disease and immune thrombocytopenic purpura and one child with hereditary spherocytosis were observed**.**

In children aged between 3-6 years, 32 had Iron deficiency anemia, 4 children had Thalassemia, 7 had acute leukemia, 3 children had Immune thrombocytopenic purpura, two children had Aplastic anemia. Immune hemolytic anemias, Anemia of chronic disease, Hereditary spherocytosis, storage disease contributed one case each.

In the age group of 6-12 years, Iron deficiency anemia presented in 47 children.6 children had acute leukemia, 5 children had Anemia of chronic disease, 3 had aplastic anemia and one child had storage disorder. In Thalassemia major mean age at diagnosis is 2.56 years ranging from 10 months to 7 years (Table 1).

CHART 5

AGE

DISTRIBUTION

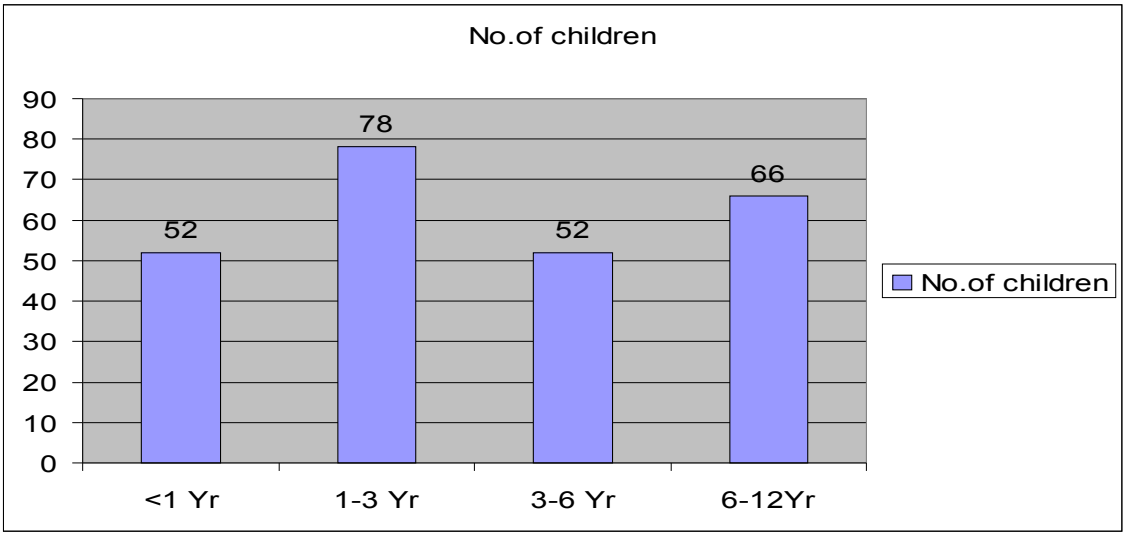


TABLE 1

RELATIONSHIP BETWEEN AGE AND ETIOLOGY

AGE	IDA	THAL	AL	IHA	ACD	HS	SD	ITP	MA
<1 YEAR	35	6	1	7		1			2
1-3 YEARS	63	4	5	1	3	1		1	
3-6 YEARS	32	4	7	1	1	1	1	3	2

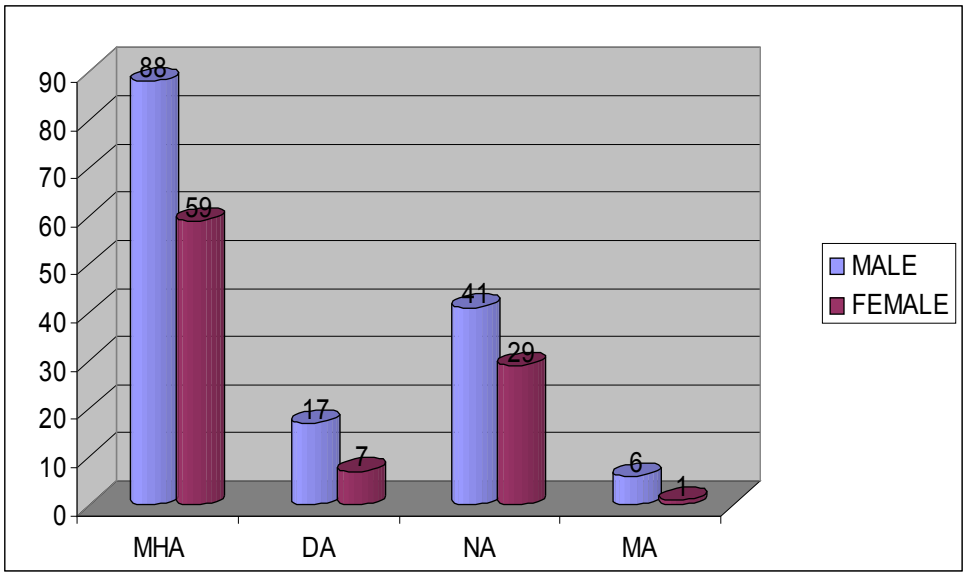
6-12 YEARS	47	3	6	1	5		1		3
	177	**17**	**19**	**10**	**9**	**3**	**2**	**4**	**7**

SEX

Out of 248 children studied 152 children were boys (61%) and 96 children were girls (39%). The Boys; Girls ratio was 3;2. In boys, Microcytic hypochromic anemia accounted for 88 children (57.8%), dimorphic anemia-17 children (11.1%), normocytic anemias-41 children (26.9%) and macrocytic anemia-6 children (3.9%). In girls, Microcytic hypochromic anemia was present in 59 children. Dimorphic anemia-7 children, normocytic anemia-29 children, macrocytic anemia-1 child (CHART 6).

CHART 6

RELATIONSHIP BETWEEN SEX AND MORPHOLOGY



PRESENTING SYMPTOMS

Out of 248 children, 204 children complained of easy fatiguability. Worm infestation was present in 88 children. PICA was present in 29 children. Glossitis was present in 10 children whereas koilonychia could be appreciated in 4 children only. Bleeding diathesis such as bleeding gums and presence of petechiae and purpura was seen in 12 children.

SIGNS

Pallor was the most common sign in our study and it was seen in 220 cases. Hepatosplenomegaly was seen in 140 children. Lymphadenopathy seen in 16 children and jaundice was present in 20 children.

LABORATORY FINDINGS

HEMOGLOBIN

Mean Hb values in our study is 6.7 gms. Anemia was graded into mild, moderate and severe anemia by Hb levels.

Mild anemia was seen in 10 children (4%), moderate anemia in 112 children (45.1%) and severe anemia in 126 children. (CHART 7) (50.8%) In boys, severe anemia accounted for 77 children (50.65%) whereas moderate and mild anemia were seen in 67(44.07%) and 8 children (5.2%) respectively. In girls, mild anemia seen in 2 children (2.08%), moderate anemia was seen in 45 children (46.8%) and severe anemia in 49 children (51.04%) (Chart 8).

In microcytic hypochromic anemias, severe anemia was seen in 88 children and moderate anemia 56 children,3 children of mild anemia. In dimorphic anemias, mild anemia was present in 3 children, moderate anemia in 16 children and severe anemia in 5 children. In normocytic anemias, mild anemia and moderate anemia contributed 4 and 39 cases respectively. Severe anemia presented in 27 cases. In macrocytic anemias, moderate anemia was seen in one child, severe anemia in 6 children. (CHART 9)

CHART 7

SEVERITY OF ANEMIA

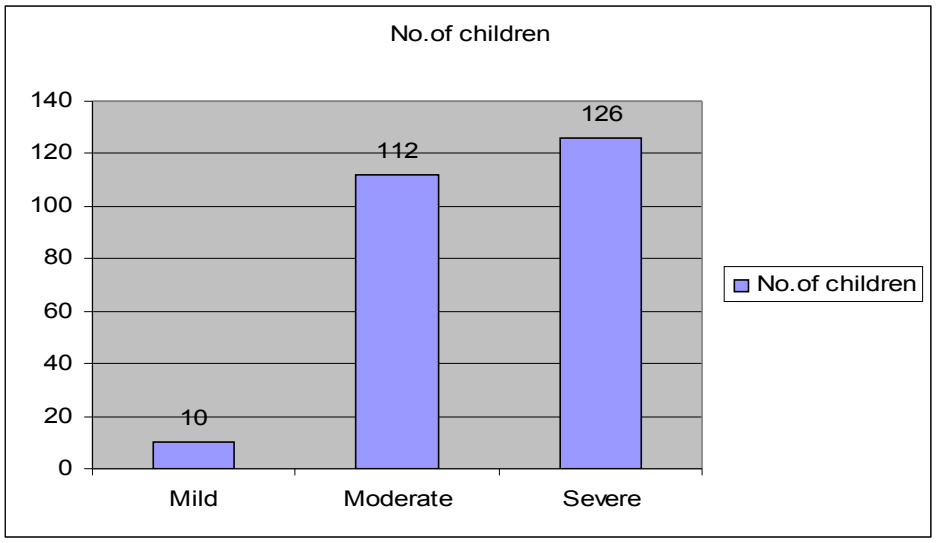


CHART 8

RELATIONSHIP BETWEEN SEX AND SEVERITY OF ANEMIA

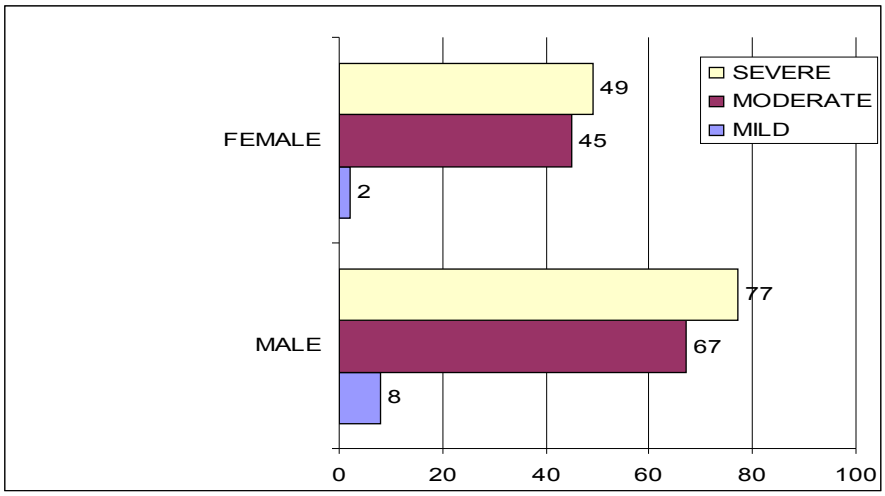
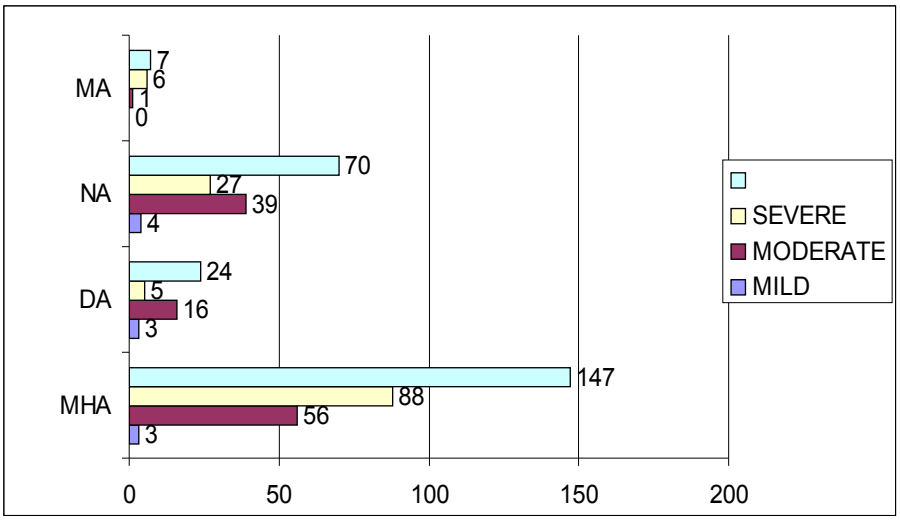


CHART 9

RELATIONSHIP BETWEEN MORPHOLOGY AND SEVERITY OF ANEMIA



TOTAL RBC COUNT

Average RBC count in our study is-3.39 million/cumm

Children with RBC count>5million/cumm-6

Children with RBC count <5 million/cumm-242

In Iron deficiency anemia average RBC count is 4.12 million/cumm

In Thalassemia Trait average RBC count is 5.12 million/cumm.

MCV

Average MCV in our study is -75.1 fl.Low MCV was seen in 139 children. Normal MCV seen in 101 children. High MCV seen in 8 children.

In microcytic hypochromic anemias, low MCV seen in 125

children, normal MCV in 22 children. In dimorphic anemias, low MCV seen in 5 children, and normal MCV seen in 19 children. In normocytic anemias, low MCV seen in 9 children, normal MCV in 59 children and high MCV in 2 children. In macrocytic anemias normal MCV was present in one child and high MCV in 6 children (CHART 10). In Iron deficiency anemia, low MCV seen in 116 children, normal MCV in 61 children. In Thalassemias and HbE only low MCV was seen.

In acute leukemias 2 children had low MCV and the remaining 17 children had normal MCV. Two children were having low MCV in ACD and 7 children with ACD had normal MCV.

Children with immune hemolytic anemia had normal MCV values in all cases. In Immune thrombocytopenia, two children had normal values and two children had low MCV. In megaloblastic and aplastic anemias all children had high MCV. (TABLE 2)

In Iron deficiency anemia average MCV was 73.24 and in other children average MCV was 79.46. p value was 0.0006 (significant).

CHART 10

RELATIONSHIP BETWEEN MCV AND MORPHOLOGY

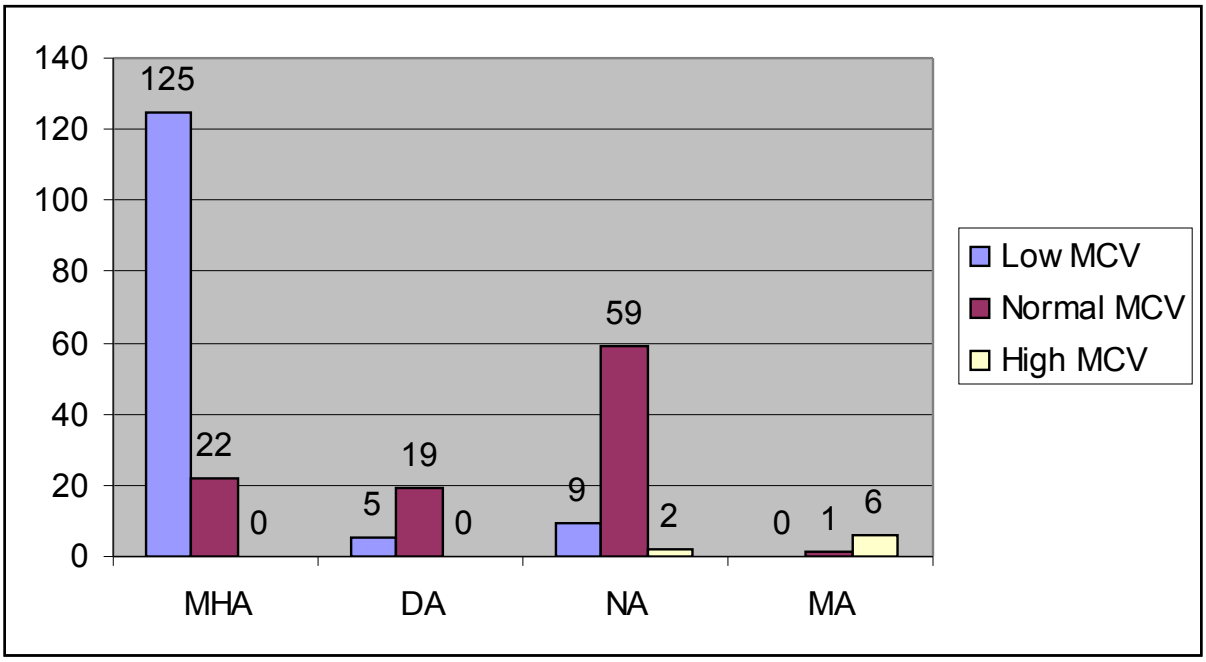


TABLE 2

RELATIONSHIP BETWEEN MCV AND ETIOLOGY

ETIOLOGY	LOW MCV	NORMAL MCV	HIGH MCV
IDA	116	61	
Thal Major	12	0	
TT	4		
HbE	1		
AL	2	17	
IHA		10	
ACD	2	7	
HS		3	
AA			7
MEG ANEMIA			
ITP	2	2	
SD		2	
	139	**102**	**7**

MCH

Mean MCH in our study was 20.4.Low MCH seen in 132 children. Normal MCH seen in 108 children. High MCH seen in 8 children.

In children with Iron deficiency anemia the average MCH was 19.18 and in other children average MCH was 23.64 .p value was 0.0001 (significant).

MCHC

Mean MCHC in our study was 27.1.Mean MCHC in children with Iron deficiency anemia was 26.07.In other children MCHC was 29.75.p value was 0.0001(significant).

Mentzer Index

Mentzer index was applied in children with microcytic hypochromic anemia. In our study, 119 children with hypochromic microcytic anemia had mentzer index >14. Mentzer index Sensitivity was 92.2%.Specificity was 86%.Youden's Index was 78.2.

RDW

Red cell distribution width was increased in 175 children and was within normal limits in 73 children. In microcytic hypochromic anemias RDW was increased in 110 children and was normal in 37 children. In dimorphic anemias RDW was elevated in 15 children and normal in 9 children. In normochromic normocytic anemias normal RDW seen in 26 children and elevated RDW in 44 children. In macrocytic anemias RDW was normal only in one child but elevated values seen in 6 children (Table 3).

In Iron deficiency anemias RDW was normal in 48 children and elevated in 129 children. In Thalassemia major RDW elevated in 9 children and normal in 3 children.50% of Thalassemia trait (2) children had elevated RDW and other two children had normal RDW. In acute leukemia, normal RDW seen in 5 children and elevated RDW seen in 14 children. 5 children with Immune hemolytic anemias and 5 children with anemia of chronic disorder had elevated RDW and the remaining children had normal RDW (Table 4).

TABLE 3

RELATIONSHIP BETWEEN MORPHOLOGY AND RDW

MORPHOLOGY	NORMAL RDW	ELEVATED RDW
MHA	37	110
DA	9	15
NA	26	44
MA	1	6

TABLE 4

RELATIONSHIP BETWEEN RDW AND ETIOLOGY

ETIOLOGY	NORMAL RDW	ELEVATED RDW
IDA	48	129
TM	3	9
TT	2	2
HbE	0	1
AL	5	14
IHA	5	5
ACD	4	5
HS	2	1
AA	1	4
MEG ANEMIA	0	2
ITP	1	3

SD	2	0

PERIPHERAL SMEAR

Microcytosis was noticed in 204 children. (82.2%) Anisopoikilocytosis was present in 107 (43.1%) children. Macrocytes seen in 133(53.6%) smears. Target cells seen in 56 (22.5%) children. Polychromatic cells seen in 26 children and nucleated RBCs were present in 36 smears. Spherocytes seen in 9 smears.Blasts seen in 18 children.

BONE MARROW

Bone marrow aspiration was done in 36 children. 19 children had hypercellular marrow with increased blasts and diagnosed as acute leukemia.12 children had hypercellular marrow with erythroid hyperplasia. In bone marrow aspirates of 2 children there was increased histiocytes and two children had increased megakaryocytes consistent with immune mediated thrombocytopenic purpura.

In one child marrow was hypoplastic.

SPECIAL STUDIES

All children were first treated with oral dose of iron (ferrous sulfate). Children diagnosed to have leukemia and other diseases were exempted from treatment. Oral iron was administered in the form of ferrous sulfate

containing elemental iron in a dose of 4mg/kg for two months. The response was monitored by measuring blood Hb levels and reticulocyte count.

Children who recovered from anemia after two months of oral iron therapy were presumed to have iron deficiency anemia. Children who failed to improve after treatment were subjected to investigations. Serum Iron, Total iron binding capacity, Serum Ferritin levels and Hb electrophoresis were done in 20 children.

3 children had low to normal Sr Iron levels with low serum ferritin and serum iron and increased TIBC. Hb electrophoresis revealed normal study.

Those children were diagnosed to have Iron deficiency anemia. 12 children had increased serum ferritin, serum iron and abnormally elevated fetal Hb levels in Hb electrophoresis. They were diagnosed to have Thalassemia major. In another 4 children mild anemia persisted and microcytosis was apparent in the peripheral smear. These children had normal serum iron and ferritin levels and high HbA2 levels in Hb electrophoresis. The diagnosis was thalassemia trait.

A 7 year old female child had normal serum iron, and low TIBC. Hb electrophoresis showed low HbA levels, high fetal Hb levels (26%) and very high HbE levels (HbE+HbA2-70%).

In our study we encountered a 4 year old boy with history of recurrent infections and profound anemia. Clinical examination showed marked pallor and mild hepatosplenomegaly. Peripheral smear study showed anisocytosis with predominant macrocytosis, normocytes and tear drop cells.

Leucopenia with lymphocytosis and marked thrombocytopenia was present. Moderate anemia was present with normal reticulocyte count.
Liver function tests were normal .TIBC was within normal limits and serum ferritin was high than normal levels.

Bone marrow was hypocellular with no megakaryocytes and increased fat spaces. Markedly decreased myeloid and erythroid precursors with megaloblastic changes were encountered. Chromosomal analysis by Mitomycin-C revealed a high frequency of chromosome breakage compared to control. The diagnosis was Fanconi's anemia.

DISCUSSION

In this present study the prevalence of anemia among children attending O.P in pediatric department, Government Rajaji Hospital is 18.1%.This incidence is similar to the studies done by Kaya et al[61] where the incidence is 20%.

In our study boys are predominant and the M;F ratio is 3:2.(M-61%, F-39%) This is similar to the study conducted by kaya et al[61] in which 67 boys and 43 girls are enrolled. The incidence of anemia among boys is 18.2%. This incidence is higher than incidence quoted by kaya et al[61].In girls the incidence of anemia is 18.0% compared to 7.5% incidence in malatya study.

In our study, mean age at diagnosis of Thalassemia major is 2.16 years which can be compared to the studies quoted in literature as 13.1 months to 2 years[13,63,32]. 9 children with Thalassemia major (75%) are aged less than 3 years and this observation is similar to the study conducted by J.Patel et al.[41]

Morphologically microcytic hypochromic anemia is the most common morphological type seen in our study accounting for 59.25% children with anemia. This finding is similar to the studies conducted by

Azmat manzoor et al[7] where the incidence is 62.9% where as it is much higher when compared to studies conducted by Kapoor D et al[45] where the incidence is 33.9%.Dimorphic anemia comprised 9.4% cases in our study. This is low when compared to the studies by Kapoor D et al[45].

Normochromic normocytic anemia is seen in 28.25% cases in our study. This finding is similar to the finding of Azmat manzoor et al[7] study where the incidence is 29.3%.

Macrocytic anemia is present in 2.8% cases in our study which is much lower when compared to 10.3% incidence quoted by Azmat manzoor et al[7].

Anisopoikilocytosis is present in 43.1% children in our study whereas Azmat study quoted the incidence as 24.8%.22.5% smears showed target cells in our study which is higher than incidence given by Azmat manzoor et al[7].

Out of 248 children, 204 children have the complaint of easy fatiguability. This confirms the observation in western literature that easy fatiguability is the most common symptom in anemia particularly Iron Deficiency anemia. PICA is present in 29 children in our study. This symptom is found in 50% children in study conducted by Crosby et al[16].

In our study iron deficiency anemia is the most commonest cause for microcytic hypochromic anemia. (129/147) accounting for 87.75% cases. This finding is similar to the studies conducted by viswanath D et al[87] where the incidence is 89% and lower than the incidence quoted by Fazal razim khan et al [7](92%).

The total prevalence of Iron Deficiency anemia among anemic children is 70% in our study. This incidence is much lower than the finding of Kapoor D et al[45] where the incidence is 88%.

Among microcytic hypochromic anemias mild anemia incidence is 2.04%, moderate anemia is 38.0% and severe anemia is 59.8%..In Thalassemia Major children all children had severe anemia which is higher when compared to 75% children in a study conducted by Patel et al[41.]

In our study, microcytosis is present in (93/126) 73.8% children with severe anemia and (72/112) 64% children with moderate anemia and 60% cases of mild anemia. This can be compared to the findings of Viswanath D et al[87] where the prevalence is 100%, 62.5%,22.5% respectively.

In children with iron Deficiency anemia, 65.5% has low MCV levels. This correlates with the literature studies of 70%[11]. MCHC levels are only elevated in 44% children. This finding correlates with literature

studies that MCV is a more sensitive index than MCHC in detecting Iron Deficiency anemia. In our study, mean MCV levels in Thalassemia major children is 65.5 fl with levels varying from 45 to 72.2fl.This correlates with levels quoted by literature[95].

Mentzer Index sensitivity and specificity is 92.2% and 86% respectively. Youden index is 78.2. This can be compared to the results shown by M.A.Ehsani et al[21] where the sensitivity and specificity is 94.6% and 95.5% and Youden's Index 90.1.Other studies by Demir et al (2002) and Urrechaga et al (2008) have quoted 82.0 and 80.9 respectively[21] (Table 5).

TABLE 5

ACCURACY OF MENTZER INDEX-COMPARISON STUDIES

STUDY	RESULTS
Demir et al (2002)	82
Urrechaga et al (2008)	80.9
Ehsani et al	90.1
Present study	78.2

RDW is elevated in 129 children out of 177 children suffering with iron deficiency anemia (72.7%) which is lower than 90% quoted by Marsh W Jr, Bishop JW et al[53].

The sensitivity of RDW in detecting cases of Iron deficiency anemia is 72.8% in our study. The specificity is 73.7%. This finding can be compared to the sensitivity and specificity of 90% and 77% quoted by Leima CS, Reis AR et al[49].

In literature it has been quoted that increased RDW is 90-100% sensitive and only 50-70% specific in diagnosing Iron Deficiency anemia[21].

RDW is elevated in 50% children with Anemia of chronic disease and 50 % cases of Thalassemia trait. T his can be compared to the studies conducted by Marsh et al[53] where 32% of Anemia of chronic disorder and 66% of Thalassemia Trait have elevated RDW.

In our study, incidence of Thalassemia Trait is 1.6% which is lower than the incidence given by Salma Sheikh et al[72].

In our studies three children with hereditary spherocytosis are studied .In all the children MCHC levels are more than 37gm% with elevated RDW. In the literature studies it has been mentioned that MCHC greater than 35.4 gm/dl and elevated RDW is suggestive of Hereditary spherocytosis.[60]

In macrocytic anemias neutropenia is present in all children in our study, which is higher when compared to studies conducted by Dr.Jagdish

et al [41] with neutropenia varying from 17-49%.Platelet count is decreased in all children in our study, but Dr.Jagdish Chandra et al[41] has put the incidence at 80%.

In our study megaloblastic anemia comprised 28% children with pancytopenia which is similar to the studies of Jagdish et al[41].Aplastic anemia accounts for 72% children with macrocytic anemias whereas Dr.Jagdish quoted 20% incidence only. The RDW in megaloblastic anemia is markedly elevated when compared to RDW in aplastic anemia where it is normal or mildly elevated. The finding correlates to the studies conducted by Buetler E, Fairbanks et al[11].

Incidence of megaloblastic anemia in our study is 0.8% which is lower than the incidence quoted by Gomber et al[29]In our study, serum ferritin levels are low in Iron Deficiency anemia children whereas it is normal or elevated in other children. This finding correlates with the studies done by Shine JW et al[76] who concludes that low serum ferritin is the best single laboratory parameter to diagnose Iron deficiency.

Hbelectrophoresis done in 20 patients shows high fetal Hb levels varying from 43% to 90%.This correlates with western literature studies where fetal Hb levels varies from 10-90% in Hb electrophoresis.

In our study we have one case of Fanconi's anemia who is a 4 year old boy. In literature it is mentioned that 75% cases of Fanconi's anemia are reported between 4-14 years.

The boy has mild hepatosplenomegaly and do not have any other congenital abnormalities mentioned in the literature. However it has been mentioned in literature that 14% cases do not have any congenital abnormalities[88]. Chromosomal analysis by Mitomycin-C revealed a high frequency of chromosome breakage compared to control.

In literature it is mentioned that random chromatid breaks are present in myeloid cells, lymphocytes, and chorionic villus biopsy samples. This chromosome damage is intensified after exposure to DNA cross-linking agents such as mitomycin C or diepoxybutane. The hypersensitivity of the chromosomes of marrow cells or lymphocytes to the mitomycin C agent is used as a diagnostic test for the condition.

Based on test result child is diagnosed to have Fanconi's anemia.

SUMMARY

The salient features observed in the present study are

1) 3381 children are referred to clinical pathology laboratory from pediatrics department for hematological investigations.
2) 614 children of them are found to be anemic.
3) Children who are undergoing treatment, who have received blood transfusions are excluded and remaining 248 children enrolled in this study.
4) Peripheral smear assessment and complete hemogram studies are done in all the children.
5) 45 children have evidence for leukemia, pancytopenia and hemolytic anemia in peripheral smear.
6) Bone marrow aspiration, osmotic fragility test, Direct Coomb's test are done in these 45 children.
7) Of these 45 children,19 children have acute leukemia,10 children have immune hemolytic anemia,5 children have aplastic anemia,4 children have immune thrombocytopenic purpura,3 children have hereditary spherocytosis, 2 children have storage disorder and 2 children have megaloblastic anemia.

8) The remaining 203 children are given oral iron therapy.

9) 174 children have responded to treatment and are presumed to have Iron deficiency anemia.

10) 29 children do not respond to treatment and are subjected to special investigations.

11) Of these 29 children, 12 children have Thalassemia Major, 4 children have Thalassemia Trait, 3 children have Iron deficiency anemia,9 children have anemia of chronic disease and 1 child has HbE disease.

12) Automated hemogram paramenters like MCV, RDW Mentzer Index are correlated with the cause for anemia.

13) The sensitivity and specificity of MCV in identifying Iron Deficiency anemia are 65.5% and 75.5% respectively.
P value is 0.0006.

14) The sensitivity and specificity of RDW are 72.8% and 73.7%
P value 0.0031.

15) The sensitivity and specificity of Mentzer Index are 92.25% and 86%.

16) Children in 1-3 years age group are commonly anaemic.

17) The most common morphological pattern of anemia observed in this study is microcytic hypochromic anemia.(59.25%).

18) Other patterns observed are Dimorphic anemia (9.6%), normochromic normocytic anemia (28.25%) and macrocytic anemia (2.8%).

19) The most common cause of anemia observed in this study is Iron deficiency anemia (71%)

20) Other causes are Acute leukemia (7.66%),Thalassemia major (4.83%), Immune hemolytic anemia (4.03%), Anemia of chronic disease (3.62%), aplastic anemia(2.01%),Immune thrombocytopenic purpura(1.61%),hereditary spherocytosis(1.2%), megaloblastic anemia (0.08%).

CONCLUSION

The diagnosis and treatment of anemia needs a stepwise approach. First in sequence and importance is to rule out the associated neoplastic diseases such as acute leukemia and aplastic anemia.

Next morphologic classification using peripheral smear assessment and automated hemogram studies are done. Children who do not have evidence for any other disease are assumed to have iron deficiency anemia and oral empirical iron therapy is given.

Finally we have to do special studies in children who do not respond to treatment to ascertain the cause of anemia.

Unfortunately majority of our population cannot afford these costly investigations and there exists huge discrepancies in the diagnosis of anemia between developed and developing countries.

Peripheral smear assessment combined with automated hemogram can provide clue regarding the exact etiology in a majority of children. However in a minority serum Iron studies and hemoglobin electrophoresis are needed to identify the cause.

To mark the finale of this thesis of mine, I conclude with certain amount of confidence that iron deficiency anemia is the most common

cause of anemia in children. The automated hemogram with peripheral smear study is beneficial and sufficient for the morphological subtyping and diagnosis of anemia for the majority of children attending the out patient department at Govt Rajaji Hospital, Madurai.

BIBLIOGRAPHY

1. Ahmal Fayez Bakr et al. European Journal of Pediatrics 2006;165(7);442-5
2. Anderson B et al. Investigations into the Euglena method for assay of Vit B12 in serum. J Clin Pathol 1964;17;14-26
3. Antille R et al. body iron stores decrease in boys during pubertal development and soluble transferring receptor –feritin ratio as an indicator of iron status. Pediatr Res 1997;41;224-8.
4. Auerbach AD et al. Susceptibility of Fanconi's anemia fibroblasts to chromosomal damage by carcinogens .nature 1976;261;494-496
5. Aulokh R.sohi I.Singh T.kakkar N RDW in diagnosis of ID with MHA Indian J pediatr 2009 Mar 76(3) ;265-8
6. Arvind Lal et al-The Thalassemia saga express health care
7. Azmat Manzoor, Muhammed Tayyib, Tahira Tasnim. Anemia in school children Pak Postgrad Med J Mar 2003;14(1):44-7. Department of Pathology, Postgraduate Medical Institute, Lahore.
8. Begum Y et al. Quantitative assessment of erthropoeisis ;blood 1993;81(4) 1067-1076

9. Bessman JD,Feinstein D. Quantitative anisocytosis as a discriminant between iron deficiency and thalasemia minor.
10. Bessmann JD et al ,Improved classification of anemia by MCV and RDW.Am J clin Pathl1983;8013;322-326
11. Beutler E, Fairbanks VF: The effects of iron deficiency, in: Iron in Biochemistry and Medicine II, edited by A Jacobs, M Worwood, p 393. Academic Press, New York,
12. Bothwell TH.the diagnosis of Iron deficiency..N Z MED J 1966;65;880-883
13. Cao A,Galanello R,Rosatelli MC et al. Clinical experience of management of thalassemia ;the Sardinian experience .Semin Hemato1996;1;66-75
14. Christima Ullrich et al. New iron deficiency test shows promise for infants Clinical lab products nov 2005(1-4)
15. Cook JD.The measurement of sr transferring receptor .Am J med Sci 1999;18;269-76.
16. Crosby WH.Pica JAMA 1976;235;2765
17. Cunningham TM,Hemoglobin E in Indochinese refugees .West J

Med 1982;137(3)186-190

18. Dagg JH.Goldberg A,lockhead A.Value of erythrocyte protoporphyrin in the diagnosis of latent iron deficiency .br j Haematol 1966;12;326-330.
19. Dallmann PR, Diagnosis of anemia and iron deficiency ;Analtic and biologic variations of laboratory tests .Am J Clin Nutr 1984;39(6)937-941
20. Dimitriou H, Stiakaka E, et al.Acta Pediatr 2000;89;1169-73
21. M.A. Ehsani, E. Shahgholi, M.S. Rahiminejad, F. Seighali and A. Rashidi A NewIndex for Discrimination Between Iron Deficiency Anemia and Beta-Thalassemia Minor:Results in 284 Patients Pakistan Journal of Biological Sciences Year: 2009 | Volume: 12 | Issue: 5 | Page No.: 473-475
22. El sahn F, Salam S. East Mediterr Health J 2000;6;1017-25
23. England Jm. ward SM, Doun MC. Microcytosis, anisocytosis and the red cell indices in Iron deficiency.Br j Hematol 1976;34(4)589-597.
24. Fairbanks VF.et al. Homozygous HbE mimics β thalassemia minor without anemia(or) hemolysis, am J hematol 1980;8(1) 109-115

25. Fairbanks VF.et al. Homozygous HbE mimics β thalassemia minor without anemia (or) hemolysis, am J hematol 1980;8(1)118-121.
26. Fielding J.O' Shaughnessy MC,Brunstrom GM.Iron deficiency without anemia.Lancet 1965;2;9-12
27. Fischer S, MCV .Arch Intern Med 1983;143;282-283
28. Glader BE, Hemolytic anemia in children Clin Lab Med 1999;19;87-111
29. Gomber S.Kumar ,Rusia u et al .Prevalence and etiology of nutritional anemias in early childhood in an urban slum.Indian J Med Research 1998;107;269-273
30. Goodnough LT, Skikie B,Brugnan C, Erythropoeitin, iron, and erythropoeisis, Blood 2000;96;823-33.
31. Gordon .N. Iron Deficiency and the intellect bruin dev 2003;25;3-8
32. Gupta PK, Saxena R, Karan AS, Choudhury VP. Red cell indices for distinguishing macrocytosis of aplastic anemia and megaloblastic anemia. Indian J Pathol Microbiol2003; 46: 375-377.
33. HamT. Hemoglobinuria Am J Med 1955;18;990-1006
34. Hamilton PJ.davidson RL. the The interrelationship and stability of coulter s determined blood indices .J.Clin pathol 1973;23(9)700-705

35. Herbert V et al.Measurement of folic acid in serum Blood 1960;15;228
36. Heinrich HC.Iron deficiency without anemia .Lancet 1968;2(7565);460
37. Hoffbrand AV et al .method of assay of red cell folate activity and the value of the assay as a test for folate deficiency .j Clin Pathol 1966;19;(1)17-28
38. Hulthen L. LinstedtG. Et al, Effect of a mild infection on sr ferritin concentration Eur J clin Nutr 1998-;52;376-9
39. Indian journal of medical sciemces Pub date 01/02/08Author-sinha N; Deshmukh p; gary B
40. Itano M.CAP comprehensive chemistry Serum iron survey Am J Clin Pathol 1978;70;516-522
41. Dr.Jagdish Chandra et al. Supplementary information submitted to the SCN working group on micronutrients Report 2006; Vitamin B12 and folate deficiency; megaloblastic anemia and beyond.
42. J.Patel, A.Patel, A.Kaur & V.Patel; Prevalence of Haemoglobinopathies in Gujarat-A cross sectional study. The Internet Journal of Hematology 2009 Volume 5;1-9

43. J.Trop Pediatr 1999 Aug ;45(4);221-5 ;the pattern of common anemia among saudi children
44. Johnson cs,Teges C. Beutler E. thalassemia minor Routine erythrocyte measurements and differentiation from Iron deficiency .Am J Clin pathol 1983 80(1) 31-36
45. Johnson I et al. Measurement of red cell folate with selenofolate radioassay J Clin Pathol 1978;31(1);47-49
46. Kapoor D. Agarwal KN, Iron status of children aged 9-36 months ; ICDS project Indian Pediatrics 2002 Feb 136-44
47. Koller me, Romslo, Finne PH, et al. The diagnosis of iron deficiency by erythrocyte protoporphyrin and serum ferritin analysis .Acta pediatr scand 1978;67;361-66
48. Lau ks et al. Measurement of sr B12 level using radioisotope dilution and coated charcoal .Blood 1965;26;202-214
49. Lima cs,Reis AR, Grotto HZ, Saad ST,Costa FF ; Comparison of RDW and a red cell discriminant function incorporating volume dispersion for distinguishing iron deficiency from beta thalassemia trait in patients with microcytosis;Sao Paulo Med J.1996 Sep Oct;114(5):1265-9

50. Linderbaum J. Megaloblastic anemia and neutrophil hypersegmentation .Br J Hematol 1980;44(3);511-513.
51. Lipshitz ,Cook JD et al. Sr ferritin as an index of iron stores N Engl J med 1974-290;1213-1216
52. Marsh WL, Jr Keenig HM, The lab evaluation of microcytic red cells Crit rev Clin lab Sci.1982;16(3);195-254
53. Marsh WL Jr,Bishop Jw, Darcy TP. Evaluation of RDW Department of laboratory medicine ,naval hospital, San Diego, California. Hematol.Pathol.1987;1(2);117-23
54. Martin PL et al. The anemias Philadelphia; Lippincott 1994:165
55. Mathew D;observations on estimation of Vit B12 .Clin Sci 1962;22;101-111
56. Matsunaga AT. Lubur BH, Hemolytic anemia in new born.Clin Perinatal 1995;22;803-828
57. McClure S.Custer E.Bossman JD. Improved detection of early iron deficiency in non-anemic subjects .JAMA 1985;253(7)1021-1023
58. Mentzer WC .Jr .Differentiation of Iron deficiency from Thalassemia Trait Lancet 1973;882
59. Michaels L.A cohen AR, Zhao H et al. Screening for hereditary

spherocytosis by use of automated erythrocyte indices J.Pediatr 1997;130;957-960

60. Michaels LA et al. Screening for HS by erythrocyte indices J Pediatr 1997;130;957-960
61. Mine Kaya et al, Iron Deficiency anemia among school students in malatya, Turkey A cross sectional study.
62. M.L.Hermiston W.C. Mentzer Pediatr Clin N Am 49(2002)
63. Modell CB, Berdoukas VA. The clinical approach to thalassemia. New York;Grune &Stratton,1984.
64. Molla et al JDMA -1992 may 42(5);118-121
65. Olivares M.et al. Anemia and iron deficiency disease in children .br med B cell 1999;55;334-43.
66. OrlicD Ultrastructural analysis of erythropoeisis; In Gordon AS,ed, Regulation of hematopoeisis Vol 1,New york;Appleton-century-crofts;1970;271
67. Padmanabhan A et al ;Ann trop Paediatrics -2001 Mar21/17;45-9High prevalence of microcytic anemia in omani children –a prospective study.
68. Panick .V.G-6Pd Part 2 Trop Asia clinical hematology

1981;10;800-814

69. Rath .C.Finch CA .Sternal marrow hemosiderin 1948;3;81-86
70. Ross DC et al.α thal is associated with increased soluble transferring receptor levels Br J Haematol 1998;103;365-69
71. Sahibzada Syed Masoodus Syed, Muhammad Saeed Razi, Saleh Muhammad, Sohail Amjad, Ahmed Affifi. Frequency of Anemia in Pediatric out patient department Pak Paed J Mar 2004;28(1):35-6.
72. Salma Shaikh, Khalique Rehman Shaikh, Manzoor Memon. Prevalence of iron deficiency and thalassaemia trait in children at Pediatric Department of L.H.U Biomedica Jan - Jun 2003;19:11-7.
73. Sasaki MS et al.A high susceptibility of Fanconi's anemia to chromosomal breakage by DNA cross linking agents cancer Res;1973;33;1829-1836
74. Schwartz;E;IDA; nelson textbook of pediatrics 16th edition 1469-71
75. Serden MA et al.The role of EP in IDA in children J trop Pediatr 2000;46-323-6
76. Shine JW Am Fam physician .1997 May 15;55(7);2455-62 Related articles, Links microcytic anemia.
77. Soed.S.K. and U.russia (1986);Ann of Nat Acad of Med.Sci.India

22(4) 235

78. Statland B,Winkel P.Bokland H Variation of serum Iron conc in healthy men,clin Biochem 1976;26-29
79. Statland BE,Wikel P. Relationship of day-to- day variation in serum Iron conc Am J Clin Pathol 1977;67(1) ;84-90.
80. Stohlman F.Kinetics of erythropoeisis; In; Gordon AS; regulation of hematopoeisis. Vol 1,New york;Appleton-century-crofts;1970;317
81. Tisman G et al.B12 dependence of cell uptake of sr folate. Blood 1973;41(3)'465-69
82. Tooze J.davies HG; The occurrence and possible significance of Hb in the chromosomal regions of mature erythrocyte nuclei.J cell Biol 1963;16;501-511
83. Urtanen MJA.et al. Higher concentrations of serum transferring receptor in children Am J Clin Nutr 1999;69;259-60
84. Verloop MC. Iron depletion without anemia ;a controversial subject .Blood.1970;36;657-671
85. Venter A,Vender Pol J.L East Mediterr Health J 1995; 64-70
86. Verma et al;Indian Paediatrics 1998 Dec 35(12);1181-6
87. ViswanathD, Hegde R,Murthy V,nagashree, ShahR Red cell

distribution width in the diagnosis of iron deficiency anemia. Indian J pediatr.2001 Dec:68(12);1117-9.

88. Weatherall DJ.Clogg JB. The thalassemia syndromes ;Oxford Blackwell science 2001

89. Werkman HP et al The short term .Iron rhythm ; Clin Chem Acta 1974;53;(11);65-68

90. WHO (1968) techn .Rep.Ser.No.40

91. Wiana FH Jr et al. Discrimination between ID and ACD Am J Clin Pathol 2001;15(1)112-118

92. William's Hematology 7th edition ;page 413

93. Wintrobe TENTH edition page 12.

94. Wintrobe TENTH edition page 951

95. Wintrobe ELEVENTH edition p1334

96. Worwood M. Sr Ferritin CRC Crit Rev Clin labSci.1979;10(2); 171-204

97. Young NA, Alter BP: Aplastic Anemia: Acquired and Inherited. WB Saunders, Philadelphia, 1994.

PROFORMA

NAME : **AGE/SEX :**
ADDRESS :

COMPLAINTS :

H/O PRESENT/ PAST ILLNESS :

GENERAL EXAMINATION :
NUTRITION
STATURE
PALLOR
ICTERUS
PURPURA
ECCHYMOSIS
LEG ULCERS

SYSTEMIC EXAMINATION :
HEPATOMEGALY
SPLENOMEGALY
LYMPHADENOPATHY

INVESTIGATIONS :

Hb%

TC

DC

ESR

RBC COUNT

PCV

URINE - ALBUMIN
SUGAR
DEPOSITS

MOTION - OVA

CYST
OCCULT BLOOD

RDW

MCV

MCH

MCHC

RETICULOCYTE COUNT

OSMOTIC FRAGILITY TEST

DIRECT COOMB'S TEST

Sr PROTEINS **ALBUMIN**

GLOBULIN

Sr BILIRUBIN **TOTAL**
DIRECT
IN DIRECT

Sr FERRITIN
Sr IRON
Sr TIBC

PERIPHERAL SMEAR EXAMINATION

BONE MARROW EXAMINATION

Hb ELECTROPHORESIS

MISCELLANEOUS :

DIAGNOSIS :

MASTER CHART – EXPLANATION OF THE CODES USED

COMPLAINTS

1- Easy fatiguability

2- PICA

3- Fever

4- Loss of appetite

5- Loss of weight

6- Jaundice

7- Loose stools

8- Failure to thrive

9- Bleeding gums

10- Abdominal distension

11- Breathlessness

12- Worm in stools

CF - Clinical Features

1- Pallor

2- Hepatomegaly

3- Splenomegaly

4- Lymphadenopathy

5- Icterus

6- Pedal edema

7- Bossing of skull, prominent cheeks

8- Malnurished

9- Purpuric spots

10-Glossitis

PS- Peripheral Smear

1- Anisopoikilocytosis

2- Hypochromic microcytes

3- Macrocytes

4- Normochromc normocytes

5- Elongated cells

6- Target cell

7- Polychromatic cell

8- Nucleated RBCs

9- Spherocytes

10-Sickle cell

11-Tear drop cell

12- Fragmented cell

BM- Bone marrow findings

1- Normocellular marrow

2- Hypercellular marrow

3- Hypocellular marrow

4- Micronormoblastic erythroid maturation

5- Megaloblastic erythroid maturation

6- Normoblastic erythroid maturation

7- Normal pattern of myeloid maturation

8- Active myeloid maturation

9- Normal megakaryocytes

10- Increased megakaryocytes

11- Low megakaryocytes

FINAL DIAGNOSIS – ABBREVIATIONS

IDA	Iron Deficiency Anemia
AA	Aplastic Anemia
GD	Gaucher's Disease
AL	Acute Leukemia
TM	Thalassemia Major
ACD	Anemia of Chronic Disease
ITP	Immune Thrombocytopenic Purpura
IHA	Immune Hemolytic Anemia
HS	Hereditary Spherocytosis
TT	Thalassemia Trait
HD	HbE Disease
MOA	Megaloblastic Anemia
FA	Fanconi Anemia